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Total synthesis of the natural isoprenylcysteine carboxyl methyltransferase inhibitor spermatinamine

José García, Raquel Pereira, Angel R. de Lera *

Departamento de Química Orgánica, Facultade de Química. Universidade de Vigo, 36310 Vigo, Spain

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ABSTRACT

The first total synthesis of spermatinamine, an inhibitor of isoprenylcysteine carboxyl methyltransferase (Icmt) with a bromotyrosine–spermine–bromotyrosine dimeric structure is described.

Post-translational modifications of eukaryotic proteins are important for their cellular localization and function.[1] The covalent attachment of isoprenoid groups to proteins that contain CAAX motifs (C, cysteine; A, aliphatic amino acid; X, any amino acid) has gained considerable attention recently because it is the initial step of cell signaling pathways that control diverse biological processes.[2,3] C_{15}-farnesyl or C_{20}-geranylgeranyl groups are the most common isoprenoids enzymatically attached to the cysteine at or near the carboxy terminus of the CAAX-containing protein[4] through a mechanism that depends upon the sequence of amino acids of the X residue,[5] and in general involves Zn^{2+}.[6] Three different prenyltransferases,[7] namely farnesyltransferase (FT), geranylgeranyltransferase 1 (GGT1) and geranylgeranyltransferase 2 (GGT2) mediate transfer of isoprenoids in humans, although these enzymes have also been found in other eukaryotes including vertebrates, insects, plants, protozoa and fungi.[4]

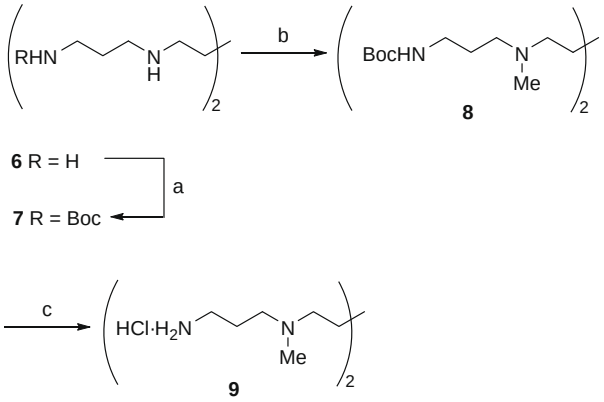
One of the best-studied CAAX motif-containing proteins is the Ras GTPase superfamily (39 members)[8–10] which can be prenylated at the cysteine side chain with either C_{15} (X small aminoacid: Ala, Ser) or C_{20} (X = Leu) prenyl chains. *S*-prenylation of CAAX proteins is followed by additional post-translational modifications in sequence involving *S*-palmitoylation of neighbouring cysteine groups, specific endo proteolytic cleavage of the AAX residue (by Ras-converting enzyme Rce1, a farnesyl-*S*-Ras endoprotease),[11–13] and methylation of the carboxyl cysteine residue (by isoprenylcysteine carboxyl methyltransferase, Icmt).[11,14,15]

Hyperactivation of Ras is thought to contribute to carcinogenesis and tumour progression.[3,16] Inhibition of (some of the) steps that covalently modify the Ras oncogene with a prenyl group prior to its recruitment to the plasma membrane are attractive targets for drug development.[10] Given the limited success of farnesyltransferase inhibitors (TFIs), attention has been turned to other proteins on the Ras processing pathway as new anticancer drug targets.[10] In fact, drugs that inhibit Icmt (methotrexate and cysmethynil **1**, Fig. 1)[17] have shown promising antitumour properties in tissue cultures and animal models.[18–20]

Aplysamine 6 **2**[21] and spermatinamine **3** (Fig. 1),[22] isolated from extracts of the Verongida sponge *Pseudoceratina* sp., were the first natural products shown to inhibit Icmt. More recently, bioassay-guided purification of *Hovea parvicalyx* extracts[23] led to isolation and identification of new β-hydroxychalcone derivatives (**4** and **5**, Fig. 1) with Icmt inhibitory activity.

Spermatinamine **3** (IC_{50} = 1.9 μM for Icmt) is representative of a group of antitumour natural products extracted from Verongidas that biosynthetically derive from the condensation of bromotyrosine and spermine.[22] Due to its potential as a lead to discover more potent Icmt inhibitors with better bioavailability, we undertook the total synthesis of spermatinamine **3** and analogues. The first synthesis of aplysamine 6 **2** reported recently[24] prompted us to disclose our approach to spermatinamine **3**.

The synthesis of spermatinamine **3** utilizes commercially available *O*-methyl-L-tyrosine **10** and spermine **6** as suitable precursors



Scheme 1. Reagents and conditions: (a) BOC-ON, THF, 0 °C, 2 min, 80%; (b) 37% HCHO, $NaBH_3CN$, CH_3COOH, EtOH, 25 °C, 12 h, 96%; (c) 4 M HCl, 1,4-dioxane, 25 °C, 4 h, 87%.

* Corresponding author. Tel.: +34 986812316; fax: +34 986811940.
E-mail address: qolera@uvigo.es (A. R. de Lera).

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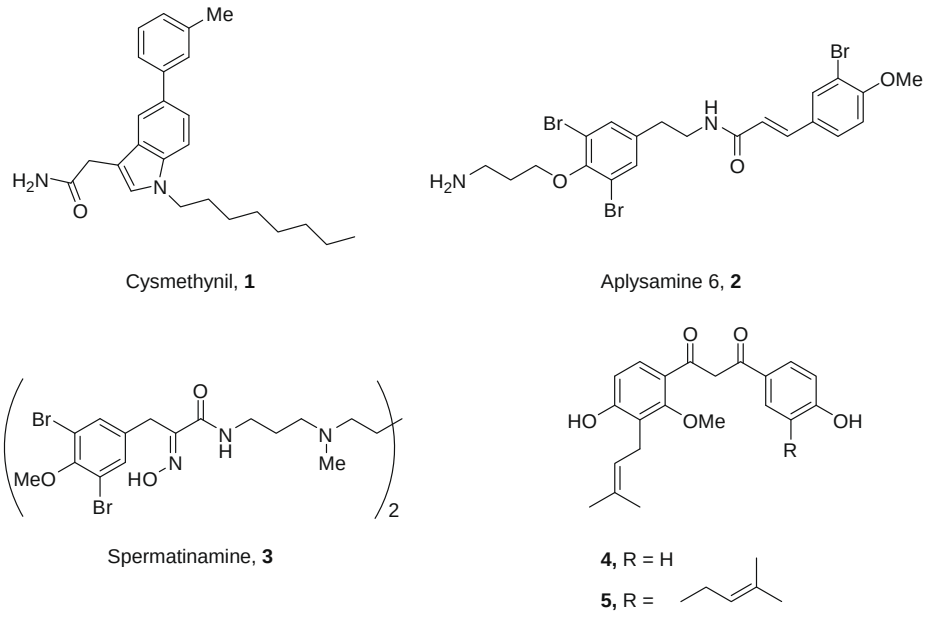
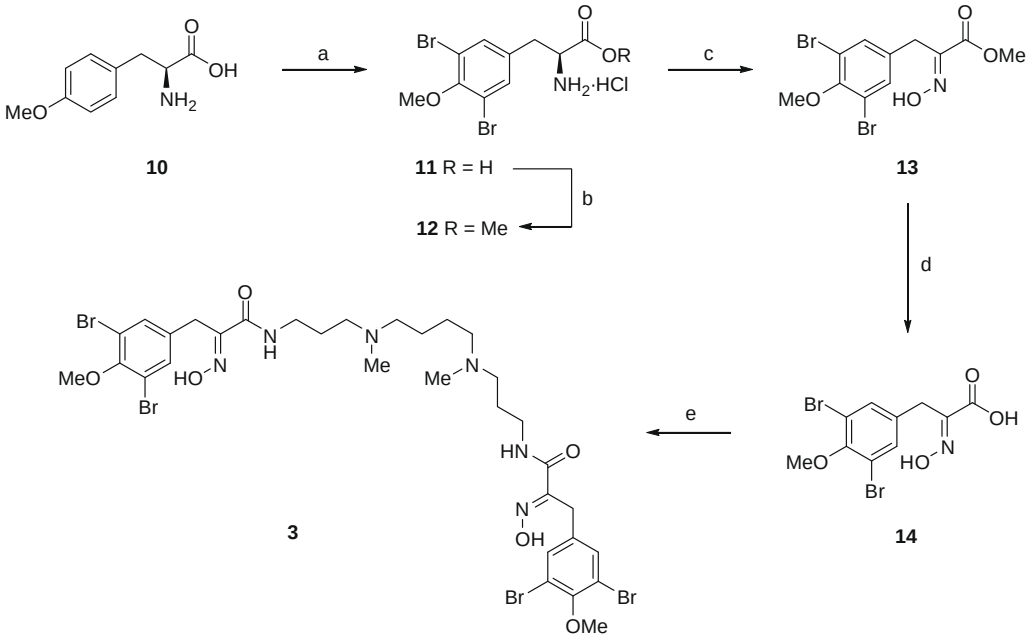


Figure 1. Chemical structure of Icmt inhibitors.

to build the bis-bromotyrosine–spermine skeleton. After the initial attempts to protect the primary amino groups of spermine **6** using Boc_2O failed, protected amine **7** could be obtained by treatment of **6** with BOC-ON[25] in THF. Subsequently methylation of **7** by reductive amination gave **8** in good yield (Scheme 1). Final deprotection of the terminal amino group with 4 M HCl afforded hydrochloride **9** in excellent yield (Scheme 1).

As shown in Scheme 2, the synthesis of 3,5-dibromo-*O*-methyl-L-tyrosine **11** was carried out by direct bromination of commercial *O*-methyl-L-tyrosine **10** with bromine in a water/37% hydrochloric acid mixture at 0 °C.[26] Treatment of acid **11** with thionyl chloride in MeOH afforded amino ester **12** which was then converted to oxime ester **13** using sodium tungstate and 30% aqueous hydrogen peroxide.[27] Only freshly opened bottles of hydrogen peroxide afforded good to excellent yields in the oxidation of amino ester **12**. *E*-Oxime ester **13** was obtained as the only geometric isomer and its configuration was confirmed by comparison with that of spermatinamine **3**.[22] After saponification, oxime acid **14** was condensed with methylated spermine **9** following the procedure described by Hoshino[28] which uses *N*-hydroxyphthalimide and dicyclohexylcarbodiimide (DCC) as activating agents to couple oxime acids with amines, to give spermatinamine **3** in 50% overall yield (Scheme 2).[29] The spectroscopic data of synthetic **3** matched those of the natural specimen.[22] Other coupling agents (HOBt,



Scheme 2. Reagents and conditions: (a) Br_2, 37% HCl, H_2O, 0 °C, 15 min, 56%; (b) $SOCl_2$, MeOH, 80 °C, 4 h, 85%; (c) $Na_2WO_4{\cdot}2H_2O$, EtOH, H_2O_2, H_2O, 25 °C, 4 h, 68%; (d) LiOH, (1:1) THF/H_2O, 25 °C, 14 h, 95%; (e) **9**, *N*-hydroxyphthalimide, DCC, 1,4-dioxane, 25 °C, 8 h, 50%.

N-hydroxysuccinimide, EDC) failed to afford amide **3** with the unprotected oxime group, thus lengthening the sequence to reach the target.[30,31]

Although as indicated[22] spermatinamine **3** does not exhibit the required drug-like pharmacokinetic properties, the synthetic approach described herein should pave the way for the preparation and biological evaluation of novel analogs that might expand the number of marine natural product-inspired anticancer drugs[32] in particular as inhibitors of relatively unexplored targets, such as Icmt.[17]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.087.

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29. *N*-Hydroxyphthalimide (0.07 g, 0.41 mmol) and DCC (0.08 g, 0.41 mmol) were added to a solution of acid **14** (0.15 g, 0.41 mmol) in dioxane (1 mL). After the mixture had been stirred for 8 h at room temperature, a solution of amine **9** (0.06 g, 0.20 mmol), Et_3N (0.11 mL, 0.82 mmol) and MeOH (1 mL) was added. The reaction mixture was stirred for 12 h, the solvent was removed in vacuo and the residue was purified by column chromatography (SiO_2, 95:5 CH_3OH/NH_4OH) to afford 0.09 g (50%) of **3** as a white solid, mp: 172–173 dec. (MeOH/Et_2O). ^1H NMR (DMSO-d_6, 400.13 MHz) (data for monomer): δ 8.18 (t, J = 5.4 Hz, 1H, NH), 7.45 (s, 2H, 2H2′), 3.76 (s, 2H, 2H3), 3.74 (s, 3H, OCH_3), 3.17 (q, J = 5.6 Hz, 2H, 2H1″), 2.4–2.3 (m, 4H, 2 × CH_2), 2.16 (s, 3H, NCH_3), 1.60 (quintet, J = 6.5 Hz, 2H, 2H2″), 1.4–1.3 (m, 2H, CH_2) ppm. ^{13}C NMR (CD_3OD, 100.62 MHz) (data for monomer): δ 165.5 (s), 153.9 (s), 152.1 (s), 137.5 (s), 134.6 (d, 2×), 118.7 (s, 2×), 61.1 (q), 58.0 (t), 56.1 (t), 41.6 (q), 38.7 (t), 28.9 (t), 26.7 (t), 24.8 (t) ppm. IR: ν 3500–3100 (br, NH and OH), 2944 (m, C–H), 2864 (w, C–H), 1662 (s, C=O and C=N), 1524 (s), 1470 (s), 1421 (m), 1259 (m), 1000 (s), 752 (m), 740 (m) cm^{-1}. MS (ESI^+): m/z (%) 931 ($[M+1]^+$ [$^{81}Br_3$] [^{79}Br], 37), 929 ($[M+1]^+$ [$^{81}Br_2$] [$^{79}Br_2$], 54), 927 ($[M+1]^+$ [^{81}Br] [$^{79}Br_3$], 39), 492 (100), 406 (99). HRMS (ESI^+) calcd for $C_{32}H_{45}{}^{81}Br_3{}^{79}BrN_6O_6$, $C_{32}H_{45}{}^{81}Br_2{}^{79}Br_2N_6O_6$ and $C_{32}H_{45}{}^{81}Br^{79}Br_3N_6O_6$ $[M+1]^+$ 931.0075, 929.0092 and 927.0109; found: 931.0051, 929.0082 and 927.0094.
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